"A STUDY OF LIPOPROTEIN (a) ESTIMATION IN STROKE PATIENTS WITH DIABETES MELLITUS"

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A STUDY OF LIPOPROTEIN [a] – ESTIMATION IN STROKE PATIENTS WITH DIABETES MELLITUS"

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P.S.G. INSTITUTE OF MEDICAL SCIENCES AND RESEARCH, COIMBATORE.

CERTIFICATE

This is to certify that this dissertation "A study of Lipoprotein [a] Estimation in stroke patients with Diabetes Mellitus" is a bonafide work done by Dr. Santhosh Jeyaraj in the Department of Medicine PSG Institute of Medical Sciences and Research, Coimbatore under my supervision and guidance. The dissertation has been rectified and is re-submitted as per the University requirement.

Coimbatore :

Date:

Prof. Dr.K. Jayachandran MD.,
Professor & HOD of Medicine,
Department of Medicine,
PSG IMS & R,
Coimbatore – 641004.

Dr. S.Ramalingam MD.,
Principal ,
PSG IMS & R,
Coimbatore – 641004.

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Dr. Santhosh Jeyaraj

CONTENTS

INTRODUCTION

Strokes are one of the most common causes of mortality and long term severe disability. There is evidence that lipoprotein [a] is a predictor of many terms of vascular disease. Several studies have evaluated the association between Lipoprotein[a] and ischemic stroke.

THE STRUCTURE OF Lp[a]:

Lipoprotein[a] is an LDL like molecule consisting of an apoprotein [apo] B-100 particle attached by a disulphide bridge to apo[a] (1) APO[a] is a member of a family of "Kringle" containing proteins. Such as plasminogen, tissue platelet activates [TPA], prothrombin factor XII, macrophage stimulating factor [MSF] (2,3). Lipoprotein[a] shares high degree of identity with plasminogen (2,4).

The apo(a) gene is highly polymorphic and more than 34 different sized alleles have been identified (5) molecular weights range from 187 to 648 DC.

The accumulation of Lipoprotein[a] molecule has been demonstrated in arterial walls of human cerebral vessels (6). This affinity may be attributed to the affinity may be attributed to the tendency of apo (a) to bind to connective tissue elements such as proteoglycans,

glycosaminoglycans, and fibronectin (7). The binding process is promoted by lipoprotein lipase or sphingomyelinase (8). Lipoprotein[a] competes with plasminogen for its receptors on endothelial cells, leading to diminished plasma formation, thereby delaying clot lyses and favouring thrombosis (9).

It has been suggested that apo [a] can cause endothelial dysfunction by enhancing lipid deposition in vessel walls inhibiting fibrinolysis and modulating smooth muscle cell proliferation (10). Raised Lipoprotein[a] concentrations were a significant determinant on the extent of carotid atherosclerosis. Therefore the estimation of lipoprotein[a] might help identify patients with an increased risk of stroke (11).

Dietary modifications is thought to influence Lipoprotein[a] values. A diet rich in palm oil has been reported to reduce Lipoprotein[a] concentration by approximately 10% (12). Nicotinic acid is said to have favourable effect on Lipoprotein[a] concentration (13) (14). Statins are said to increase Lipoprotein[a]. Fibrates are said to decrease Lipoprotein[a] levels (15) (16). Optimizing weight and tight glycemic control may beneficially influence Lipoprotein(a) values in patients with type 1 and type 2. diabetes (17). This effect is linked to triglyceride metabolism which is impaired in type 2 diabetes mellitus as well as glycosylation of Lipoprotein[a] which interferes with its catabolism (17).

This study is designed to evaluate the association of Lipoprotein(a) in patients with diabetes mellitus who have suffered an ischemic stroke.

AIM OF STUDY:

The aim of this study is to investigate the serum lipoprotein [a] levels in 38 cases of stroke patients with diabetes mellitus and to determine the consistency of its association when compared with 38 statistically matched diabetic individuals as controls.

REVIEW OF LITERATURE:

Lipoprotein [a] is a circulating lipoprotein that resembles LDL cholesterol in core lipid composition and in having apo B 100 as a surface apolipoprotein. Apo [a] is bound to apo B-100 by a disulphide bond (18). Apo [a] is a glycosylated protein that resembles plasminogen and is composed of serial Kringle domain (19). The physiological function of Lipoprotein[a] remains obscure, although a role in wound healing is proposed (20). Plasma levels of Lipoprotein[a] vary widely among individuals, are generally unrelated to those of other lipoproteins and apolipoproteins (21) and appear to be highly heritable (22). Risk of cardiovascular events appear to be increased when plasma levels exceed 20 to 30 mg /dl (23).

Non genetic mechanisms which regulate serum Lipoprotein[a] levels are unknown, although levels are increased in people with renal insufficiency, nephritic syndrome, diabetes and in menopausal women.

Ethnicity plays a major role in Lipoprotein[a] levels. African Americans have higher median levels of Lipoprotein[a] than white people (24) possibly due to transcription of the apo [a] gene or increased secretion of apo [a] (25). Elevated Lipoprotein[a] levels may be more atherogenic in presence of small apo [a] size [<22 Kringle – 4 repeats] V/s larger apo [a] isoforms (26). In African Americans, high

Lipoprotein[a] levels are less likely to be associated with the presence of small apo [a] isoforms than in white people (27).

The factors influencing serum Lp(a) concentrations are given below:

1. Physiological factors
    - Age
    - Ethnic groups
    - Menopause
    - High saturated fat diet
2. Chemical compounds and drugs
    - Oestrogen
    - Progesterone
    - Growth hormone
    - Neomycin
    - Alcohol
    - Cyclosporin

3. Diseases

- Myocardial Infarction
- Renal Failure
- Nephrotic Syndrome
- Familial hypercholesterolemia

PATHOPHYSIOLOGICAL LINK BETWEEN Lp [a] AND ATHEROTHROMBOSIS:

Accumulation of Lipoprotein[a] molecules has been demonstrated in arterial walls of coronary and cerebral vessels (6). The binding process is promoted by lipoprotein lipase or sphingomyelinase. Lipoprotein[a] particles are prone to oxidative modification and scavenger receptor uptake, leading to intracellular cholesterol accumulation and foam cell formation (28) (29) which contributes to further atherogenesis. The high affinity of Lipoprotein[a] for fibrin provides a basis for frequent colocalisation in atherosclerotic plaques (30) (31).

In vitro studies indicate that lipoprotein[a] enhances the synthesis of plasminogen activator inhibitor 1 [PAI – 1] by endothelial cells. PAI – 1 is the main inhibitor of fibrinolytic system (32). Another important action of Lipoprotein[a] is it reduces activation of latent transforming

growth factor β [TGE- β] by displacing plasminogen from surface of macrophages in atherosclerotic plaques. In absence of activated TGE – β, cytokines might induce smooth muscle cell proliferation and transform these cells into more atherogenic cellular phenotype (33).

RELATION BETWEEN Lp [a] AND ATHEROSCLEROSIS :

Recent investigations have shown whether Lipoprotein[a] plays a primary or synergestic role in atherosclerosis (25) cross sectional and retrospective studies involving white men have shown increased risk of coronary and cerebrovascular atherosclerotic disease associated with plasma Lipoprotein[a] concentration greater than 80^{th} centile [>250 -300 mg/litre]. Recent prospective studies revealed a modest significant association between IHD and increased Lipoprotein[a] concentrations (34-35-36). Studies have shown that children with a positive family history of IHD commonly exhibit small apo [a] phenotypes (37) (38). This association of small apo [a] isoforms with higher Lipoprotein[a] concentrations suggests that raised Lipoprotein[a] concentration antedate the atherosclerotic process (25).

The pathophysiological correlation between Lp(a) and atherosclerosis is given below:

1. Contributes to uptake of LDL and formation of foam cells
2. Inhibits plasminogen activator and fibrinolysis leading to procoagulant tendency
3. Release of cytokines
4. Release of growth factors and smooth muscle cell proliferation
5. Increased expression of adhesion molecules
6. Endothelial dysfunction
7. Interacts with other risk factors (eg) Homocysteine

Lp [a] AND FIBRINOGEN:

Serum Lipoprotein[a] concentrations correlates well with plasma fibrinogen values in some studies (39) (40). This relationship is interesting because platelet activity is enhanced by fibrinogen (41) (42) and raised plasma fibrinogen concentrations are predictors of vascular events, both in healthy populations and inpatients with IHD (43).

There is a strong evidence that fibrinogen is an independent risk factor for ischemic atherothrombotic stroke (44). Fibrinogen values

remain raised after stroke and are associated with increased risk of recurrent vascular events (45). In patients with stroke, fibrinogen is associated with decrease in white blood cell elasticity and red blood cell deformity and an increase in plasma erythrocyte viscosity (46). Fibrinogen also promotes platelet aggregation and consumption in the ischemic area in patients with stroke (47).

In the above scenario fibric acid derivatives can decrease circulating concentrations of Lipoprotein[a] and fibrinogen (48) (49). So platelet inhibitory activity of fibric acid derivatives could be mediated via their action on lipid fractions [TG and HDL], Lipoprotein[a] and fibrinogen (50) (51).

TRIGLYCERIDE CONCENTRATIONS AND STROKE:

Postprandial hypertriglyceridemia is associated with carotid artery atherosclerosis.Various epidemiological and cohort studies have reported elevated levels of serum triglyceride as an independent risk factor for the incidence of cerebrovascular accident (52).

HDL CHOLESTEROL AND STROKE:

Inverse association between HDL and stroke risk (13). Although no association was found in Framingham study (53) in the Copenhagen study (54) a negative relation was evident.

Apart from well established risk factors for strokes [age, hypertension, diabetes, smoking, or presence of vascular disease] (55) the possibility that Lipoprotein[a] is a risk factor for ischemic stroke has been assessed in several studies (56, 57, 58, 59).

Studies supporting Lipoprotein[a] as a risk factor for stroke suggested that Lipoprotein[a] values were significantly [$p<0.001$] higher in patients with ischemic stroke compared with healthy individuals [median, 95 v 50 mg /lit]. This difference was also evident in a subgroup of subjects aged 30 to 69 yrs [$p<0.001$] . In another study it was found that TG and Lipoprotein[a] levels were increased in patients 6 months after stroke. Another study suggested that serum Lipoprotein[a] levels were increased in patients with stroke (60). Another study found that 33% patients Lipoprotein [a] values were raised but not related with cardiovascular or cerebrovascular characteristics or prognosis.

Silent cerebral infarction has also come into the limelight. A silent stroke is detected on imaging in patients with no neurological signs (61). These are predisposing factors for an overt stroke. In most cases, lacunar stokes of less than $1cm^2$ in size are detected in basal ganglia in apparently healthy elderly people (62). These lesions are associated in most reports with advanced age and hypertension and constitute a major cause of dementia (62). Silent multiple lacunar strokes were associated with

hypercoagulable state, endothelial damage and a raised Lipoprotein[a] level. In atherosclerosis risk in community study Lipoprotein[a] with stroke was investigated and Lipoprotein[a] as an independent risk factor for stroke and TIA was shown (63). This evidence indicates that Lipoprotein[a] concentration is higher in patients with atherothrombotic brain infarction then in those with brain haemmorhage or lacunar infarction (64).Patients with ischemic heart disease are at increased risk of having stroke.

ATHEROSCLEROSIS AND CEREBRAL ARTERIES:

Intracranial arteries are relatively resistant to cholesterol related endothelial damage (65,66,67). In studies primates failed to show a relationship between hyperlipidemia and development of atherosclerosis in intracranial arteries. Atherosclerotic lesions were more in carotid arteries and more extensive than those in basilar, vertebral, middle cerebral arteries. Coexistence of hypertension and hypercholesterolemia resulted in accelerated atherosclerosis of intracranial arteries in rat model.

Human necropsies have shown atherosclerosis changes in cerebral arteries make their appearance 20 years later than in the coronary arteries (68). Differences in the prevalence and extent of atherosclerotic lesions in aorta and coronary or cerebral arteries were observed between different age and race groups. Black individuals and older people tend to have

more extensive cerebral atherosclerosis (68). Pinocytic vesicles in endothelium of cerebral arteries, their rich innervation and the lower distending pressures make these vessels more resistant to hyperlipidaemia compared with coronary or peripheral arteries (68).

In a recent study Lipoprotein[a] values were associated with permanent cessation of flow and occlusive arterial thrombosis (69). Analysis of damaged arterial segments showed Lipoprotein[a] incorporation in the adventitia, media and intima. Plasma Lp[a] values correlated well with carotid atherosclerosis in subjects younger than 60 years (70). Suggestions have also come up saying that apo[a] can cause endothelial dysfunction by enhancing lipid deposition in vessels, inhibiting fibrinolysis and modulating smooth muscle cell proliferation. Measurement of Lipoprotein[a] concentrations might help identify patients with an increased risk of stroke. Endothelial vasomotor tone is also related to implication of cerebral events (71) .

FORMATION OF FOAM CELLS AND ATHEROSCLEROTIC PLAQUE:

Atherosclerotic plaques but not normal human arteries contain Lp(a). Plasminogen-like lysine binding sites present on In a similar fashion, elastase cleaves Lp(a) in the same region to form F1 fragment and mini Lp(a), which is the F2 fragment connected to the LDL particle.

In vivo studies suggest that the F2 fragment is retained within the atherosclerotic plaque and is a potential cause for the atherothrombogenic action of Lp(a),1 whereas the F1 fragments may return to the circulation. Furthermore, following intravenous administration of Lp(a), F1- derived Apo(a) fragments can be isolated from plasma and urine of humans and mice. Indeed, enzymes such as metalloproteinases and elastases present within the atherosclerotic plaque may contribute to this process and hence pathogenicity of Lp(a) by breaking it down into its F1 and F2 fragments.

MECHANISMS OF LIPOPROTEIN(a) INDUCED ATHEROGENESIS INDUCTION OF ADHESION MOLECULES ON VASCULAR ENDOTHELIAL CELLS:

Lipoprotein(a) is believed to promote atherosclerosis by a number of separate but related mechanisms. Expression of adhesion molecules, VCAM-1 and E-selectin, on cultured human coronary endothelial cells is increased in the presence of Lp(a).It also induces human vascular endothelial cells to produce monocyte chemotactic protein (MCP), a potent chemoattractant for monocytes and a key cytokine implicated in the pathogenesis of atherosclerosis. Atherosclerosis is increasingly believed to be an inflammatory disease, and recruitment of monocyte macrophages is an important early step of atheroma formation. Within

days or weeks of feeding mice a high-fat and high-cholesterol diet, monocytes can be observed adhering to the surface of endothelial cells.

The monocytes then migrate into the arterial intima .The Apo(a) molecule may play a key role in anchoring Lp(a) to the extracellular matrix within the arterial wall. Mutations affecting the lysine binding sites of kringle IV-10 of Apo(a) have been shown to decrease affinity of Lp(a) to the vessel wall. Transgenic mice expressing mini Apo(a) containing a mutation in kringle IV-10 lysine binding sites have significant reduction in fatty streak formation and Lp(a) accumulation within the vessel wall. Klezovitch *et al.* have also demonstrated that proteoglycans within the vessel wall may play an important role in Lp(a) retention within the vascular intima.In these experiments, Apo(a), via its C-terminal domain, was found to bind to the protein core of the proteoglycan decorin, a proteoglycan synthesized by vascular endothelial and smooth muscle cells and present within atherosclerotic plaques.

This binding was shown to be hydrophobic in nature and not dependent on the lysine binding sites on the Apo(a) molecule. However, the nature of the interaction between decorin and intact Lp(a) was an electrostatic binding of the glycosaminoglycan (GAG) portion of decorin to the ApoB100 of Lp(a). This decorin-Apo(a) interaction has recently been proposed by Klezovitch *et al.* as an explanation for preferential

vessel wall retention of Lp(a) over LDL. Within the diseased arterial wall, Lp(a) probably undergoes oxidative, proteolytic, and lipolytic changes induced by enzymes present within an atherosclerotic plaque, such as metalloproteinase, elastase, sphingomyelinase, and phospholipase. Oxidative modification by malondialdehyde, for instance, produces avid Lp(a) uptake by human monocyte-macrophages.

Cholesterol loading of macrophages also results in marked enhancement of Lp(a) and Apo(a) internalization and degradation, revealing a lipid-driven mechanism for Lp(a) foam cell formation. Incubation of bovine aortic smooth muscle cells with Lp(a) in the presence of lipoprotein lipase and sphingomyelinase lead to massive aggregation of Lp(a) on the surface of these cells, whereas coincubation with chondroitin ABC lyase prevented this aggregation, suggesting a key interaction with cellular proteoglycans. Moreover, coincubation of Lp(a)-coated smooth muscle cells with mouse peritoneral macrophages led to formation of lipid-laden macrophages on the surface of these cells with disappearance of visible Lp(a) aggregates. This could be an important interaction between Lp(a), smooth muscle cells, and macrophages, leading to foam cell and plaque expansion.

Enhancement of expression of cell surface adhesion molecules could be an important mechanism of Lp(a)’s atherogenecity. Other

investigators have shown that Lp(a) enhances expression of ICAM-1 in cultured human umbilical vein endothelial cells (HUVEC), although a similar effect with VCAM or E-select in has not been found. Moreover, neutralizing transforming growth factor beta (TGF) antibodies enhanced ICAM expression in HUVECs, while addition of recombinant TGF_inhibited the enhancement of ICAM-1 expression in Lp(a)-treated HUVECs, suggesting that enhancement of ICAM-1 expression by Lp(a) could in part be due to inhibition of TGF. This TGF mediated effect of Lp(a) has been well documented both in vitro and in vivo in animal models and in human subjects by Grainger *et al*. and provides a theoretical basis for Lp(a) effects on smooth muscle proliferation within the vessel wall.

PLAQUE INFLAMMATION AND INSTABILITY:

Novel mechanisms of Lp(a)-mediated plaque instability have been described recently. Human THP-1 macrophages produce interleukin-8 (IL-8) in the presence of Lp(a), an effect primarily mediated by the C-terminal region of Apo(a). Interleukin- 8 is a key inflammatory cytokine within atherosclerotic plaques and possesses chemotactic activity toward neutrophils, T cells, monocytes, and smooth muscle cells, while decreasing macrophage expression of tissue inhibitors of metalloproteinases. Disinhibition of metalloproteinases that cleave

Apo(a) into F1 and F2 fragments may increase inflammatory activity within plaque leading to rupture.

The expression of urokinase and urokinase receptors on monocytes is also increased in a dose-dependent manner, resulting in increased plasmin generation. Increased protease availability may have multiple effects, including facilitation of cell migration and growth within plaque. Furthermore, monocyte adhesion to extracellular matrix (ECM) is facilitated by increased expression of micro PAR and ICAM-1, receptors for vitronectin and fibrinogen, respectively. Increased monocyte adherence to ECM and enhanced plasmin and urokinase activity could be important mechanisms of Lp(a)-mediated ECM degradation and plaque rupture.

VASCULAR CELL PROLIFERATION:

The induction of human smooth muscle cell proliferation by Lp(a) was first demonstrated in vitro by Grainger *et al.*

This group showed that Lp(a) decreased generation of active TGF, an endogenous inhibitor of smooth muscle cell migration. Bovine pericytes and smooth muscle cells secreting TGF have also been shown to inhibit endothelial cell migration and repair of a denuded portion of a vessel in vitro. Antibodies to TGF abrogated the above-mentioned

inhibition, as did inhibitors of plasmin formation. This TGF effect is mediated by inhibition of plasminogen activation at the cell surface with subsequent inhibition of plasmin-mediated TGF activation. Furthermore, inhibition of TGF activation has been observed in Apo(a) transgenic mice and in human subjects with elevated Lp(a). Apo(a) transgenic mice have been shown to have threefold less active plasmin and significantly less active TGF_within the aortic wall than normal mice. Although the total TGF concentration was similar in sera from Apo(a) transgenic and normal mice, the proportion of total TGF in active form was significantly lower in the serum of Apo(a) transgenic mice. This TGF mechanism of dysregulated growth induced by Lp(a) is an attractive hypothesis for its effects on plaque growth.

INHIBITION OF NITRIC OXIDE AND ENDOTHELIAL DYSFUNCTION:

Nitric oxide (NO) has several pleiotropic antiatherogenic properties, including inhibition of T cell and smooth muscle proliferation, neutrophil adhesion, platelet activation,and reduction in endothelial permeability. It is not surprising that decreased NO synthesis has been associated with atherosclerotic lesion development. Oxidized Lp(a) induces dose-dependent reduction of inducible nitric oxide synthase (iNOS) protein expression and mRNA synthesis in lipopolysaccharide/

interferon-stimulated mouse macrophages. Dose dependent inhibition of iNOS by Lp(a) may lead to increased atherogenesis. Elevated Lp(a) levels have also been associated with impaired endothelium-dependent vasodilatation in coronary arteries.

In hypercholesterolemic children, flow-mediated dilation of the superficial femoral artery was inversely related to Lp(a),and in patients with elevated Lp(a) levels an increased vasoconstrictor response occurs after administration of L-NMMA, an NO synthase inhibitor.76 These combined effects suggest a compensatory increase in basal NO production by the endothelium in response to elevated Lp(a) levels.

MECHANISMS OF THROMBOSIS:

Lipoprotein(a) may promote a more thrombotic state by a number of mechanisms, including inhibition of the fibrinolytic system and enhancement of the tissue factor-mediated pathway.

INHIBITION OF PLASMIN GENERATION:

Apolipoprotein(a), as discussed previously, has significant structural homology with plasminogen. A varied number of cell types have been found to express cell surface receptors for plasminogen. Both intact Lp(a) and recombinant Apo(a) inhibit plasminogen binding to endothelial cells, cells, and platelets. The assembly and activation of

plasminogen on the endothelial cell surface has been studied extensively, and it is known that plasminogen binds to the surface of endothelial cells via a tissue plasminogen activator (t-PA)/plasminogen coreceptor, identified as a member of the annexin superfamily of proteins.81 In particular annexin II, which is selectively expressed on the endothelial cell surface, possesses independent binding domains for plasminogen and t-PA. Plasminogen appears to bind to the annexin receptor in a two step process, whereby the N-terminal glutamine-plasminogen is converted to N-terminal lysineplasminogen by cleavage of a amino acid preactivation peptide with subsequent activation of the receptor in the second step.

Tissue plasminogen activator binds to annexin at a separate site in close proximity to the plasminogen-binding site, leading to more efficient generation of plasmin. Lipoprotein(a) inhibits generation of plasmin on the endothelial cell surface without interfering with t-PA binding, and in a similar manner, Apo(a) inhibits plasminogen binding to annexin but has no effect on t-PA binding.

Decreased plasminogen binding on the cell surface may therefore create an antifibrinolytic state. In addition, plasminogen activation by both streptokinase and t-PA has been shown to be impaired in the presence of Lp(a), and mice transgenic for Lp(a) are resistant to t-PA-mediated lysis of artificially induced fibrin thrombi.

The mechanism for this action is believed to be in competition with plasminogen for binding to fibrin. It is interesting that plasmin catalyzes the binding of Lp(a) to immobilized fibrinogen and fibrin. The antifibrinolytic effect of Lp(a) is primarily defined by the size of the Apo(a) polymorphs, which display heterogeneity in their fibrin-binding activity.94 The affinity of each isoform depends on its size and plasma concentrations, with smaller size isoforms displaying higher affinity binding to fibrin.

The population most at risk for thrombosis, therefore, appears to be that possessing a predominant low molecular weight phenotype with high affinity for fibrin. Moreover, the Lp(a) phenotype (i.e., affinity for fibrin) may be more important as a determinant of risk than the actual plasma concentration of Lp(a).

INCREASED EXPRESSION OF PLASMINOGEN ACTIVATOR INHIBITOR:

Endothelial cell synthesis of plasminogen activator inhibitor- 1 (PAI-1) is also increased by Lp(a).In cultured human endothelial cells, Lp(a) enhanced PAI-1 antigen activity and mRNA expression without altering t-PA activity. In addi- tion, monocytes derived from male patients with isolated Lp(a) hyperlipidemia, compared with those from healthy donors with normal Lp(a) levels, had increased upregulation of

PAI-2 mRNA and protein.This effect was gender specific, with no difference noted among females. Monocyte expression of PAI- 2 was also increased, another potential mechanism of an antifibrinolytic effect. Together these data suggest a mechanism whereby Lp(a) inhibits fibrinolysis at the endothelial cell surface and promotes thrombosis.

INHIBITION OF TISSUE FACTOR PATHWAY INHIBITOR:

Tissue factor pathway inhibitor (TFPI) is a Kunitz type serine protease inhibitor and a potent inhibitor of the tissue factor mediated coagulation cascade. Tissue factor pathway inhibitor is present on endothelial cells, activated monocytes, and platelets. However, the endothelium is believed to be the principal site of synthesis of TFPI. Tissue factor pathway inhibitor is expressed by vascular smooth muscle cells within atherosclerotic plaques, and TFPI within atherosclerotic plaque is associated with reduced tissue factor activity within the plaque.

We have recently shown that Lp(a) binds and inactivates TFPI, potentially augmenting unopposed tissue factor (TF) effects. Lipoprotein(a) can bind and inactivate recombinant as well as cell-associated TFPI in vitro in a dose-dependent manner. The LDL portion of Lp(a) isolated by dithiothreitol (DTT) reduction and gradient ultracentrifugation did not bind rTFPI, suggesting that this portion of Lp(a) was not important for binding. Apolipoprotein(a) bound to rTFPI in

a similar concentration-dependent manner as Lp(a). It is interesting that lysine plasminogen (L-Plg) was also found to bind to immobilized rTFPI but was inhibited by nanomolar concentrations of Apo(a) demonstrating a binding affinity that was lower than that of Apo(a).

Furthermore, this dose-dependent inactivation of TFPI by Lp(a) in vitro and on endothelial cell surfaces was not affected by plasminogen. No Lp(a) dose dependent binding was seen when mutated forms of TFPI lacking the K3 domain or C terminus were immobilized instead of full length rTFPI demonstrating the importance of the C-terminal region of TFPI for this interaction.

The binding of Apo(a) to rTFPI was shown to be lysine dependent and was inhibited by epsilon aminocaproic acid (EACA). This significant binding and inactivation of cell associated and recombinant TFPI by Lp(a) adds a further prothrombotic layer to the pleiotropic effects of this molecule. Thus, inhibition of TFPI within plaque at the endothelial surface and in the circulation may have additive effects in promoting thrombosis at the site of plaque rupture.

VASCULAR TISSUE AND CIRCULATORY EFFECTS:

Discovery of new mechanisms suggest that the effects of Lp(a) within the vessel wall might be more relevant to its pathogenicity and different from its effects on circulating blood. Such differential effects might be a potential explanation for blood Lp(a) levels often not correlating with the incidence of coronary events. Cleavage of Lp(a) into potential atherogenic fragments, its retention by proteoglycans, and its induction of macrophage IL-8 expression are specific vessel wall atherogenic effects not seen to occur in peripheral blood. Moreover, Lp(a) causes increased plasmin activity within a plaque while decreasing circulating plasmin activity. These differential effects on blood and vessel wall may be additive in terms of atherothrombotic risk, potentially facilitating plaque rupture and later thrombosis on the luminal surface of the vessel.

STUDIES NOT SUPPORTING Lp [a] AND STROKE:

In a study of 90 patients with stroke or TIA of atherothrombotic origin where lipid variables were measured including Lipoprotein[a], no significant difference in Lipoprotein[a] concentrations or distribution of apo E phenotypes among patients and controls was obtained (72). In a prospective study in Finland no association was found between Lipoprotein[a] and atherosclerotic disease [myocardial infarction or

stroke] (73). A study of middle aged white physicians was also conducted in which Lipoprotein[a] samples were collected together with paired controls, matched for age and smoking habits and no association was found between plasma concentration of Lipoprotein[a] and future risk of total or thromboembolic stroke was found (74).

Even a moderate rise in circulating concentration of homocysteine is said to be associated with an increased risk of Cerebrovascular accident. There has also been evidence suggesting that Lipoprotein[a] levels and homocysteine levels when increased concomitantly can cause stroke. These will have an unwanted effect on the platelets and as well as on the coagulation profile and fibrinolysis.

Increase in homocysteine by itself causes increased platelet aggregation and predisposes to ischaemic stroke. The homocysteine concentrations are influenced by renal and Vit B12 status in patients with stroke. Homocysteine and Lipoprotein[a] act together to promote atherosclerotic activity. Clustering of all these factors increased chances of vascular events. Epidemiological studies have indicated that dyslipidemia, raised fibrinogen and increased Lipoprotein[a], increases chances of vascular events.

Plasma concentration of Lipoprotein[a] and cholesterol, triglycerides and VLDL are higher in patients with hypertension. It has

been shown that increased Lipoprotein[a] with low molecular weight apo[a] isoforms are strong and independent risk factors for strong and independent risk factors for IHD in patients with hypertension.

The data on Lipoprotein[a] concentrations in diabetes are based on smaller studies and are conflicting. Larger studies and those including apo [a] phenotype analysis suggest that Lipoprotein[a] concentrations are not different from those in patients without diabetes at are said to he moderately increased in patients with diabetes. However it has been shown that Lipoprotein[a] concentration is increased in patients with diabetes and renal impairment. Further atherosclerotic complications in patients with diabetes are associated with higher Lipoprotein[a] concentrations.

Cigarette smoking is associated with increase in LDL , TG and VLDL and lowering of HDL. No direct effect of Lipoprotein[a] in smokers has been ascertained. The effect of obesity on Lipoprotein[a] levels is also not fully proved. Whereas are study says that there is no relation between obesity and Lipoprotein[a] levels another study says that reduction in Lipoprotein[a] levels was present when weight was reduced. The mechanism by which this change has occurred is not known. Apolipoprotein E[apo E] have also been associated with strokes. The apo E4 gene is high in patients with ischemic stroke. However the apo E3 /E3

phenotype has a protective association with stroke. The apo E2 genotype is a risk factor possibly expressed through obesity, diabetes, and hypertension. Lipoprotein[a] level in stroke may rise even immediately after stroke.

Under normal condition Lipoprotein[a] level remains remarkably constant throughout life. Lipoprotein[a] level is also increased in patients with chronic stroke and myocardial infarction. Change in Lipoprotein[a] levels is based on changes in production and not on the catabolic activity. Changes in Lipoprotein[a] in cardiac disease is said to be associated with changes in other lipoprotein levels. In lacunar strokes, Lp[a] level was a not found to be elevated compared to cortical infarcts.

ROLE OF Lp(a) IN MACROVASCULAR DISEASES:

Association of Lp(a) and coronary artery disease (CAD) was first observed in 1974. The accumulated data have established it as an important inherited risk factor for the macrovascular diseases like CAD, cerebrovascular accident and peripheral vascular diseases. Several case studies have shown an association of elevated Lp(a) plasma concentrations with premature coronary atherosclerosis and myocardial infarction. Lp(a) is considered to be ten times more atherogenic than LDL-C. Relative risk of CAD is increased three –fold if the levels of

Lp(a) are more than 30mg/dl. Serum Lp(a) levels have shown to correlate well with the presence, extent, severity and score of atherosclerotic lesions on coronary angiography.

The Scandinavian Simvastatin Survival study provides independent confirmation that a high Lp(a) level is a significant CAD risk factor. In Quebec cardiovascular study, however, Lp(a) was not a significant risk factor for CAD but appeared to increase the risk associated with other lipid risk factors. Lp(a) has been noted to be an independent risk factor for peripheral vascular disease.

RELATIONSHIP BETWEEN Lp(a) AND LIPID AND NON-LIPID RISK FACTORS

Correlation co-efficients of Lp(a) ranged from 0.16 to 0.17 for total cholesterol,LDL-C,HDL-C, serum triglycerides, apo A-1, apo A- II, apo – B and truncal fat . Atherogenic risk appears to be increased when there is a cluster of lipid abnormalities. Effects of serum Lp(a) on atherogenesis are increased by high LDL-C and low HDL-C levels. Men with LDL-C values of more than 317 mg/dl and Lp(a) values of more than 30 mg/dl have a sixteen-fold increase for CAD. High levels of Lp(a) were found to increase the risk associated with hyperhomocysteinemia by

a factor of nine and a simultaneous elevation in having an odds ratio of 31 for CAD.

Lp(a) AND DIABETES

In diabetes, conflicting reports are available regarding prognostic significance of Lp(a) levels. A few studies record that it may be elevated in insulin dependent diabetes mellitus. Particularly ipatients with microalbuminuria and proliferative retinopathy show higher Lp(a) levels.South Indian non-insulin dependent diabetes patients with high Lp(a) levels, however show good correlation with CAD.

REDUCTION OF Lp[a]:

Saturated and n-3 polyunsaturated fatty acids may slightly reduce Lipoprotein[a] values. Palm oil rich diet has shown to marginally decrease the Lipoprotein[a] levels (12). Nicotinic acid is also said to have a mild effect in reducing the Lipoprotein[a] levels.

Statins is said to cause a mild increase in Lipoprotein[a] level, the fact that statins does not reduce Lipoprotein[a] is because LDL receptor does not play a major role on the catabolism of Lipoprotein[a].

ROLE OF FIBRATES:

Fibric acid derivatives exert a favourable effect on TG/HDL concentrations and also on LDL quantity and quality. Fibrates reduce fibrinogen and possibly Lipoprotein[a] values. A reduction in firbrinogen concentrations was associated with decrease in incidence of primary end points cardiac death and stroke (15). Another possibility is a fibrate induced reduction in oxidized LDL production because this form of LDL is very atherogenic. Gemifibrozil reduced stroke by 1.8% [in treated group].

Body weight and tight glycemic control has a beneficial and major impact on the Lipoprotein[a] levels in patients who are diabetics. In the diabetics the triglycerides metabolism is impaired [especially in type 2 diabetes]. Treating dyslipdemia in diabetic patients improves the lipid profile and lowers chances of vascular events.

Antihypertensive can affect plasma fibrinogen and Lipoprotein[a] values as well as lipid parameters (76) due to the above factors the choice of medication in people with those predictions of vascular events [stroke] are raised (76). Hormone replacement therapy favourably affects Lipoprotein[a] level. Thyroid replacement treatment has also shown a decline in Lipoprotein[a] levels probably due to the effect on apo[a] production or Lipoprotein[a] assembly (28).

FUTURE TRENDS:

Lipoprotein(a) is now established as a genetically determined predictor of atherosclerotic vascular diseases and in particular, CAD. High levels of this lipoprotein , particularly in Asian Indians, is a matter of clinical concern. Since it is not generally amenable to the lifestyle measures, other lipid and non-lipid risk factors must be modified to decrease the risk in those with high Lp(a) levels.

The Lipoprotein[a] undergoes more changes after its entry into the arterial wall whether these changes cause changes on vascular events is yet to be ascertained. Studies assessing risk of lipoprotein[a] must also consider the contribution of other factors like dyslipidemia, hypertension, fibrinogen and homocysteine. The contribution of above factors may also have high association with increased Lipoprotein[a] values and atherosclerosis and hence more research is required in this area.

The estimation of Lp(a) levels is a useful tool for guiding management strategy in the individuals with

1. Family history of premature CAD
2. Normal total cholesterol and evidence of macrovascular disease

3. Isolated hypertriglyceridemia

4. Those belonging to high risk ethnic group

Genetic and environmental factors controlling circulating concentration of Lipoprotein[a] need further evaluation. With the given drugs the reduction of Lipoprotein[a] might prove difficult but dealing aggressively with all other risk factors associated with Lipoprotein[a] is the plan of the hour.

MATERIALS AND METHODS:

This study was carried out in thirty-eight patients presenting with ischemic stroke who also had associated diabetes mellitus and who were admitted in the medical intensive care unit, intermediate medical care unit and medical wards of PSG Hospitals, Coimbatore.

AIM OF THE STUDY;

The aim of the study is to evaluate the lipoprotein [a] levels in 38 cases of diabetic patients who presented with ischemic stroke and to determine the consistency of its association when compared with 38 diabetic patients without stroke as controls.

INCLUSION CRITERIA:

- Patients who presented to the hospital with ischemic stroke and who were known diabetics.
- The diagnosis of cerebral infarction was confirmed by clinical signs and symptoms, history of the disease and cerebral computerized axial tomography.
- Type II diabetes mellitus had been previously diagnosed.

EXCLUSION CRITERIA:

Patients with any of the following were not eligible for the study,

1. Past history of coronary artery disease.
2. Abnormal renal function.
3. Smokers.
4. Old Cerebrovascular accident.
5. Known Hypertensive.

DETERMINATION OF LIPOPROTEIN[a]:

Blood samples for the control group was taken after 12 hrs of overnight fasting under aseptic precautions.

Blood samples for the ischemic stroke patients were taken before administering heparin for the patient under aseptic precautions.

Lipoprotein[a] level was measured by the enzyme immunoassay using a monoclonal antibody against lipoprotein[a].

PROFORMA FOR THE STUDY:

Lipoprotein [a] in stroke patients with diabetes mellitus / Control.

Name : Age : Address:

Ip.No: Sex :

Date of Admission : Date of Discharge:

Presenting Complaints: Others:

Examination:

Bp: Pulse: Rhythm:

Examination of CNS:

Examination of other systems:

Any complications in 1st 24 hrs:

INVESTIGATIONS:

CT Brain : ECG : RBS: Urea:

Electrolytes: Creatinine:

Urine Routine: HbA1C:

Chest X-ray[bedside]: CBC:

RESULT AND ANALYSIS

Sex and age distribution

The sex and age of all the subjects are given in table 1.

TABLE 1

SEX AND AGE OF THE SUBJECTS IN THE STUDY GROUP AND THE CONTROL GROUP

S.No	Study group		Control group	
	Sex	Age (yrs.)	Sex	Age (yrs.)
1.	MALE	60	MALE	45
2.	MALE	60	MALE	50
3.	MALE	52	MALE	39
4.	FEMALE	71	MALE	55
5.	MALE	65	FEMALE	50
6.	MALE	48	FEMALE	58
7.	MALE	50	MALE	37
8.	MALE	39	MALE	40

9.	MALE	63	FEMALE	45
10.	FEMALE	69	MALE	62
11.	FEMALE	70	MALE	55
12.	MALE	59	FEMALE	54
13.	MALE	52	FEMALE	69
14.	MALE	47	MALE	65
15.	MALE	53	MALE	55
16.	MALE	67	MALE	63
17.	FEMALE	60	MALE	62
18.	MALE	64	MALE	70
19.	MALE	66	MALE	64
20.	MALE	69	MALE	61
21.	MALE	64	MALE	52
22.	MALE	49	MALE	57
23.	MALE	57	MALE	63

24.	MALE	46	FEMALE	55
25.	MALE	63	MALE	52
26.	MALE	74	FEMALE	47
27.	MALE	74	FEMALE	42
28.	FEMALE	76	FEMALE	57
29.	MALE	58	MALE	60
30.	MALE	55	MALE	58
31.	MALE	58	MALE	60
32.	MALE	48	MALE	62
33.	MALE	52	MALE	51
34.	MALE	64	MALE	49
35.	MALE	68	MALE	45
36.	FEMALE	67	MALE	37
37.	MALE	70	FEMALE	54
38.	MALE	74	MALE	59

The subjects involved in the study were from both the sexes and the age of the subjects ranged from 35 – 80 years. The number, sex distribution , age range and the mean age of the subjects belonging to the study group and the control group are given in **table 2**.

TABLE -2

NUMBER, SEX, AGE RANGE AND MEAN AGE OF THE SUBJECTS

Groups	Number of subjects	Sex		Age range (yrs.)	Mean age ± SD
		Male	Female		
Study	38	32 (84.2)	6 (15.7)	35-80	63.18±8.62
Control	38	28 (73.6)	10 (26.3)	35-80	56.55± 9.47

Figures in parenthesis indicate percentage of subjects

There was an equal distribution of subjects in both the groups (n=38).The percentage distribution of male subjects was higher in both the groups compared to that of the female subjects. The mean age of the subjects in the study group was found to be higher (63.18 years) compared to that of the control group (56.55 years) subjects.

TABLE – 3

SERUM LIPOPROTEIN [a] LEVELS OF THE STUDY SUBJECTS

[DIABETIC WITH ISCHEMIC STROKE]

[NORMAL VALUE < 30 mg/dl]

S.NO	Serum lipoprotein[a] levels (mg/dl)
1	62.8
2	40.9
3	51.7
4	90.8
5	38.4
6	20.3
7	64.6
8	56.8
9	88.5
10	64.8
11	50.5
12	18.3
13	80.2
14	16.2
15	53.0
16	76.8
17	55.8
18	48.6
19	66.0

20	36.8
21	57.4
22	42.0
23	30.0
24	26.0
25	27.5
26	42.4
27	38.0
28	31.0
29	24.0
30	29.6
31	32.0
32	31.8
33	52.4
34	38.4
35	36.2
36	28.0
37	29.6
38	33.4

Mean ± SD = 45.03 ± 19.19 mg/dl

TABLE- 4

SERUM LIPOPROTEIN [a] LEVELS OF THE CONTROL SUBJECTS [DIABETES]

S.NO	Serum lipoprotein[a] levels (mg/dl)
1	21.6
2	38.5
3	28.6
4	8.0
5	15.5
6	36.8
7	5.6
8	40.6
9	28.6
10	22.5
11	18.2
12	53.2
13	20.2
14	12.7
15	33.8
16	10.0
17	24.8
18	30.2
19	12.8

20	14.0
21	24.4
22	26.7
23	30.6
24	34.0
25	28.4
26	26.6
27	20.6
28	18.8
29	26.0
30	32.6
31	28.6
32	24.8
33	30.0
34	12.2
35	14.4
36	15.6
37	16.6
38	12.2

Mean ± SD = 23.66 ± 9.40 mg/dl.

TABLE- 5

COMPARISON OF THE SERUM LIPOPROTEIN (a) LEVELS BETWEEN THE STUDY AND CONTROL GROUPS

GROUPS	NUMBER OF SUBJECTS	MEAN ± SD	't' value	Level of significance
STUDY	38	45.03±19.19	5.54	p<0.001
CONTROL	38	23.66±9.40		

DISCUSSION:

The mean level of Lipoprotein [a] obtained in patients with stroke and diabetes mellitus was 45.03±19.19 mg/dl and that of the control group was 23.66±9.40 mg/dl.

On comparing both the groups, it was found out that the differences were found to be statistically very significant (t=5.54) and the level of significance was $p<0.001$.

Maurus marques de Almedia Holanda et al in their study conducted in 60 patients had found out that Lipoprotein[a] levels are significantly higher in ischemic stroke patients than in controls. They concluded saying that a reduction of Lipoprotein[a] levels was important in clinically managing patients with stroke.

Van Kooten and colleagues assessed the Lipoprotein[a] levels in 151 patients admitted with acute cerebral ischemia, they found out that about 33% of the patients had a significantly elevated plasma lipoprotein [a] level, but this was not associated with stroke characteristics or prognosis.

Nagayama et al in a case control study investigated lipoprotein[a] values in patients and found out that Lipoprotein[a] levels was an independent risk for ischemic stroke and not for lacunar stroke.

Peng et al studied the relation between lipids, apo E genotypes and risk of ischemic stroke. 180 patients were enrolled in this study. 90 patients had experienced acute ischemic strokes and 90 were healthy individuals. The study concluded that serum Lipoprotein[a] concentrations and apo E 4 genotype were prominent lipid predictors for ischemic stroke in addition to more established factors such as hypertension, family history of stroke and cigarette smoking.

In the atherosclerosis risk in communities ARIC [study the association of Lipoprotein[a] with stroke was investigated in 15160 people. In this study, Lipoprotein[a] was found to be an independent risk factor for strokes and transient ischemic attacks. Blacks and whites participated in this study and Lipoprotein[a] levels was not found to have any racial differentiation [i.e. Lipoprotein[a] associated stroke morbidity was not influenced by race]. This evidence proves the elevated level of Lipoprotein[a] is higher in atherothrombotic brain infarction than in those with brain haemmorhage or lacunar infarction.

In a study done by Jurgens and Koltringer the Lipoprotein[a] values were significantly higher in patients with ischemic stoke compared with healthy individuals.

Vavernova et al investigated Lipoprotein[a] value in 45 patients with stroke and their first degree relatives. They reported that Lipoprotein[a] values were genetically conditioned in patients with ischemic strokes.

However there have been few studies which demonstrated no relation between Lipoprotein[a] and atherosclerotic disease to quote a few studies.

Alfthan et al in a study of 7424 men and women concluded that no relation was there between Lipoprotein[a] and stroke.

Ridker PM, et al in a study of 14916 people concluded saying that no association between baseline plasma concentration of Lipoprotein[a] and future risk of all types of stroke was found.

However to find out in totality whether Lipoprotein[a] is an independent risk factor or not many studies with large numbers of people with proper inclusion and exclusion criteria will have to be done to come to a final conclusion.

SUMMARY AND CONCLUSION:

This study was carried out on 38 patients with diabetes mellitus who presented with ischemic stroke and who were admitted in the medical intensive care unit, intermediate medical care unit and medical wards.

Lipoprotein [a] was estimated by using the enzyme immunoassay using a monoclonal antibody against the Lp[a].

The lipoprotein [a] levels in this study [ischemic stroke with diabetes mellitus] group were found to be significantly [$p<0.001$] higher than those of the control group. The mean values of Lp(a) for patients with diabetes mellitus and ischemic stroke was 45.03±19.19 mg/dl and that of the control group was 23.66 ± 9.40 mg/dl.

Literature review in connection with the study revealed many studies which demonstrated the independent effects of increased lipoprotein [a] as a cause for ischemic stroke irrespective of diabetes mellitus. Many small and large studies have demonstrated the hazardous effects of increased lipoprotein [a] in patients with stroke.

The present study showed that patients with ischemic stroke with diabetes mellitus had higher lipoprotein [a] levels when compared to the control group [diabetic patients]. This goes to show that lipoprotein [a] is

an independent risk factor for stroke, and measures have to be taken to lowers the level of lipoprotein[a].

A diet rich in palm oil has been reported to reduce Lipoprotein[a] concentrations by 10% (77).

Fibrates also have an effect in reducing the Lipoprotein[a] level.

Hormonal replacement therapy in women was found beneficial in reducing Lipoprotein[a].

The findings of this study has clearly brought to light the significance of Lp(a) as an independent risk factor for the incidence of cerebrovascular disease.

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